THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 53, No. 1

Printed in U.S.A.

ULTRASTRUCTURAL CHANGES IN BUCCAL AND PALATAL MUCOSA OF ZINC DEFICIENT RATS*

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ABSTRACT

Ultrastructural changes due to a zinc-low diet (.6 ppm) were investigated in buccal and palatal mucosa of weanling rats kept on the experimental diet for periods of 3–11 weeks. Whereas parakeratosis of the entire cheek section was a consistent finding, parakeratosis of the palate was rare and never involved the whole specimen. Comparison of the fully developed parakeratotic lesions showed in both cheek and palate absence of experimental changes in lamina propria, basal and lower spinous cells. Experimental changes in the upper cell layers of buccal epithelium were increased concentrations of RNP particles, mitochondria and endoplasmic reticulum. Tonofilaments, KHG and membrane-coating granules (MCG) showed no significant changes.

In the upper cells of palatal epithelium there were no changes in concentrations of RNP particles, mitochondria or endoplasmic reticulum, but there were decreased concentrations of tonofilaments and MCG and virtual absence of KHG. The experimental keratin layer of both tissues showed persistence of nuclei and persistence of all cytoplasmic organelles except MCG. In the palate the presence of cytoplasmic organelles was limited to the deeper half, but in the cheek it extended over the entire keratin layer. In both tissues, desmosomes occupied a higher proportion of cell border in experimental animals than in controls.

Follis *et al.* in 1941 described the occurrence of parakeratosis in rats given a zinc-low diet, and considered this response as a unique effect of the deficiency of a trace element (1). The authors also observed regional differences in the response of orthokeratinizing epithelia to zinc deficiency. They noted that there was no change in the skin of paws, ears, and tail, while epidermis of the trunk and the mucosa of the esophagus and of two regions of the oral cavity showed parakeratosis (1).

Since parakeratosis is a prominent finding in diseases of the skin and oral mucosa, induction of zinc deficiency has recently been proposed as an experimental method for the study of such diseases (2, 3). Alvares and Meyer also proposed it as a method for the study of regional differences in lining epithelia (2). Using a moderate degree of deficiency (1.8 ppm), they showed a gradient of susceptibility in the oral cavity of the rat. Mucosa of the cheek in the region facing the molars was most susceptible; mucosa of the hard palate in the region of the molars was at the opposite extreme, showing no experimental changes at all.

The present study, using more severe zinc deprivation (approx. .6 ppm), showed that circumscribed regions of parakeratosis occur in palatal mucosa also and occasionally involve the entire thickness of the keratin layer. The purpose of this report is to compare the ultrastructural changes in the fully developed buccal and palatal parakeratotic lesion.

MATERIAL AND METHODS

Twenty-two male, weanling albino rats (Simonsen) weighing between 50 and 60 grams were divided into an experimental group of 14 animals and a control group of eight. The experimental group was given a powdered, purified diet (Table I) containing approximately 0.5 ppm of zinc, and distilled water containing approximately 0.09 ppm of zinc. The control group received the same diet with 20 ppm of zinc added as the carbonate, and tap water containing approximately 0.28 ppm of zinc.

The animals were housed in pairs in stainless steel cages with mesh bottoms. The food was placed in ceramic dishes and the water in glass bottles with stainless steel mouth pieces. Both food and water were obtainable ad libitum. The animals were kept on their diets for 3–11 weeks according to the schedule shown in Table II. Five experimental animals died during the 11 week period. One control animal was discarded because of an overerupted incisor.

At sacrifice, the animals were anesthetized

This work was supported by PHS grants DE-02342 and GRS FR 5309 and NRC Grant DA-166.
Received October 4, 1968; accepted for publication January 20, 1969.
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with ether and decapitated. Buccal mucosa was taken from the region facing the molars at the level of the occlusal plane; palatal mucosa was taken from the molar region midway between the midline and the gingiva. Tissue for electron microscopy was fixed in cold 1% osmium tetroxide for 30 to 45 minutes, dehydrated in a graded series of ethanol and embedded in Durcupan—ACM (Fluka A.G.). Sections were cut on an LKB Ultratome I, using glass knives. The sections were collected on collodion-coated copper grids, stained with a 5% aqueous solution of uranyl acetate and lead citrate (5) and examined with a Philips EM-75D and an RCA-EMU-3-H microscope, at magnifications not exceeding 12,000 diameters. Tissue for light microscopy was fixed in 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

The electron micrographs were scrutinized for experimental changes in the following structures: nuclei and nuclear membranes, cell borders and desmosomes; RNP particles, endoplasmic reticulum and mitochondria; tonofilaments, membrane-bound round bodies (MCG) and keratohyaline granules (KHG).

The occurrence of experimental changes in concentration was tested for RNP-particles, endoplasmic reticulum and mitochondria, and for tonofilaments, KHG, and MCG. This was done as follows: for each of six successive epithelial layers (basal, lower spinous, upper spinous, granular, deeper and superficial keratin) a large number of pairs of electron microscope micrographs from experimental and control animals were compared with respect to the seeming concentration of a given organelle. The two members of each pair were then classified according to the greater or lesser concentration of this organelle. A definite experimental increase of the organelle was recorded if the group showing greater concentration was found to contain only or predominantly micrographs from experimental animals. A slight increase was recorded if a clearcut but not overwhelming majority of the micrographs showing greater concentration was found to derive from the experimental animals. An experimental decrease was concluded from the reverse results.

The percentages of cell border occupied by desmosomes in the granular and keratin layers were measured in electron micrographs selected on the basis of sharpness of cell outlines and of desmosomes. In the cell segments where all desmosomes were clearly defined, the length of the plasma membrane was obtained by applying a flexible string to the border and measuring the length used. The desmosomes in the segment were then measured with calipers. In cheek granular layer, 208 μ total length of border were measured in controls, 325 in experimental animals; in the keratin layer 325 in control and 604 in experimental animals. In palate granular layer, 234 μ were measured in controls, 252 in experimental animals; in the keratin layer 192 in controls and 252 in experimental animals.

TABLE I

*Composition of diet**

Albumin	17%
Dextrose	58%
Cellulose	15%
Lard	5%
Salt mix†	4%
Vitamin-dextrose mix‡	1%

* Modification of Forbes and Yohe (4). Compounded by Nutritional Biochemicals Corp., Cleveland, Ohio.

† From Forbes and Yohe (4). Composition by parts: $CaHPO_4$, 708; NaCl, 101; K_2CO_3, 136.3; $MgCO_3$, 37.4; $FeSO_4 \cdot 7H_2O$, 10.8; $MnSO_4 \cdot H_2O$, 3.266; $CoCl_2 \cdot 6H_2O$, 1.080; $CuSO_4 \cdot 5H_2O$, 1.633; NaF, 0.216; KI, 0.108.

‡ Vitamin Fortification Mixture, Nutritional Biochemicals Corp.

TABLE II

Experimental schedule

Weeks on diet	No. of animals killed	
	Control	Experimental
3	1	1
4	2	3
8	2	3
11	2	2

RESULTS

A. Gross Findings

Decreased growth rates, restlessness, alopecia and skin lesions were characteristic findings in the experimental animals. Of the five animals which died while on the experimental diet, three died during the seventh week, one during the eighth and one during the tenth. The control animals showed no differences from animals fed a standard laboratory diet with respect to rates of growth or light microscopic and ultrastructural morphology.

B. Light Microscopic Findings

The major finding was the change from the complete orthokeratinization in the controls to parakeratinization in the experimental animals.

1. *Cheek* (Fig. 1a, b). All experimental cheek specimens showed parakeratinization, and this change extended over the entire section. The experimental keratin layer showed a marked increase in thickness. The degree of thickening was variable in animals killed at different time periods as well as in those killed at the same time.

2. *Palate* (Fig. 2a, b). Parakeratosis was also noted in the palatal epithelium of all experimental animals, but differed in three ways from the changes in the buccal epithelium. First, in no case did the entire specimen demonstrate parakeratosis. The areas affected were only those between rugae; the rugae maintained an orthokeratinized horny layer extending anterior and posterior to the crests. Second, parakeratinization did not often involve the entire thickness of the keratin layer. Frequently, only the lower layers possessed nuclei; sometimes nuclei persisted only in the upper layers. Presence of nuclei in the superficial keratin did not seem to be dependent upon the length of time on the diet. Third, areas that showed parakeratosis did not show thickening of the keratin layer.

C. Electron Microscopic Findings

The ultrastructural changes occurring during cell maturation in keratinizing epithelia have frequently been reported (6, 7, 8, 9, 10). Buccal and palatal mucosa of the present control animals resembled the homologous regions in the mouse in all essential features (10).

1. *Cheek.* No consistent differences between control and experimental animals were noted in the lamina propria, the region of the basement membrane, the basal cells, or the lower spinous layers.

Spinous cells (Fig. 3a, b). In the cells that had reached the upper portion of the spinous layer, the experimental animals had greater numbers of RNP particles and mitochondria than the controls. A diminution in number of these organelles compared to the basal cells characterizes normal rodent buccal epithelium at this level. This diminution was present in the controls, but not in the experimental specimens. The amount of fibrillar material appeared equal in both groups. In both, a more dense distribution than in the basal cells was noted. MCG in typical frequency occurred in the peripheral portions of the cells in the controls, and in similar number in the experimental animals.

No differences were present in nuclei, cell borders, or smooth- and rough-surfaced endoplasmic reticulum.

Granular layer (Fig. 4a, b). Experimental animals possessed higher concentrations of RNP particles, mitochondria and endoplasmic reticulum than the controls. In the controls, the concentration of these organelles was greatly diminished compared to that in the spinous cells; in the experimental animals, the decrease was much less marked. No appreciable experimental differences were noted in tonofilaments, MCG, or KHG. In both groups of animals, tonofibrillar material was present in the same amount as in the spinous cells, and the tonofilaments persisted as discrete structures, except for those associated with desmosomes. As in all layers, the tonofilaments were organized into loose bundles. In both groups, there were more MCG in the granular than in the spinous layer. KHG were present in slightly smaller number in the experimental animals, but were otherwise identical in both groups. They were small and rounded in the lower granular cells and largest in the most superficial. As in mouse buccal mucosa, they were always associated with RNP particles but not with tonofilaments. In the upper granular layers they were frequently located adjacent to mitochondria and endoplasmic reticulum.

No experimental differences in nuclei were demonstrable, and the nuclear membrane was present in both groups. The percentage of cell border occupied by desmosomal attachment plaques averaged 14 $\pm$ 1.4 (SEM) in experimental animals and 15 $\pm$ 2.1 in controls.

Keratin layer (Figs. 5a, b; 6a, b; 7a, b). In the horny layer, control and experimental animals showed obvious differences (Fig. 5a, b). The transition from the uppermost granular cell to the lowest keratin cell in the control animals was characterized by (1) a less corrugated outline and flatter shape of cells; (2) a sudden, marked increase in the opacity of the plasma membrane; (3) the persistence of a few vesicular structures and a few KHG, but the loss of nuclear material, RNP particles, MCG, KHG and most cell organelles. Persisting KHG usually had depressions in their surface and vesicles resembling mitochondrial residues were located within the depressions (Fig. 6a). Above

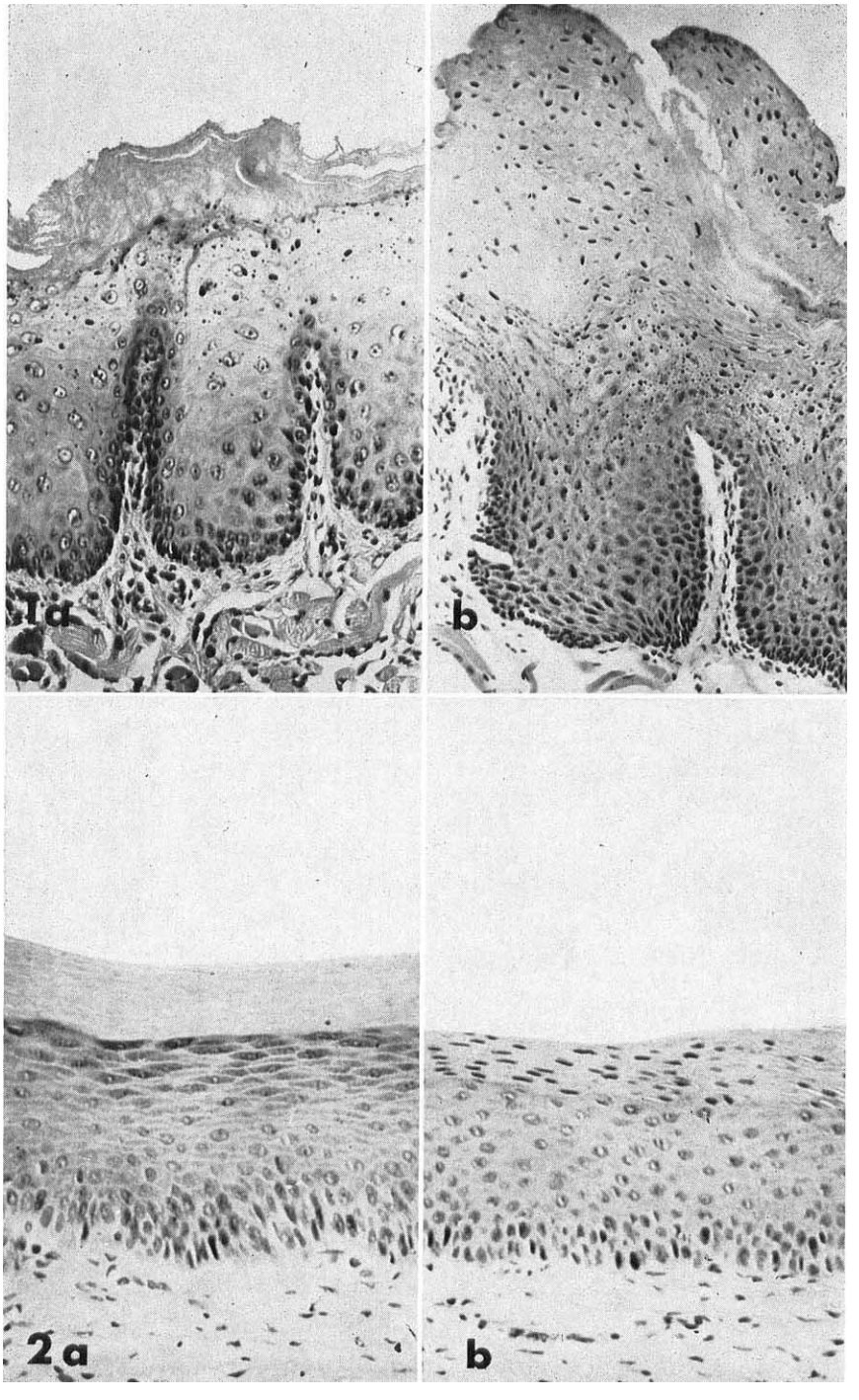
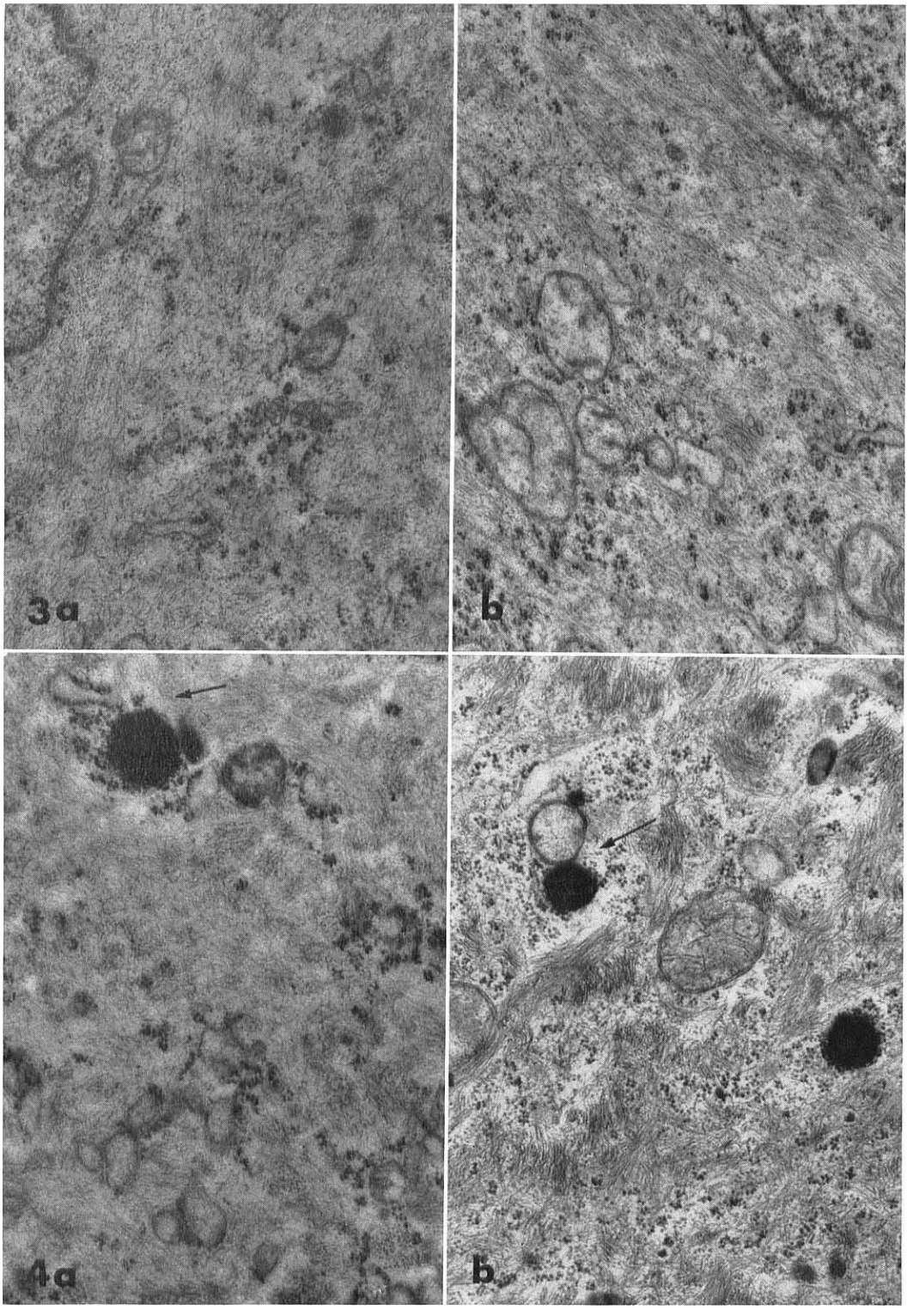


FIG. 1a and b. H and E stained sections of buccal mucosa from a control animal (a) and an experimental animal (b) sacrificed at eight weeks. Note the absence of nuclear remnants in the keratin layer of the control animal and the increased thickness of the parakeratotic horny layer in the experimental animal, × 200.

FIG. 2a and b. H and E stained sections of palatal mucosa from a control animal (a) and an experimental animal (b) sacrificed at eight weeks. Note the absence of nuclear remnants in the keratin layer of the control animal, and the retention of nuclei in the horny layer of the experimental animal, × 290.



FIGS. 3 AND 4.

the lowest layer, KHG or their remnants were seldom seen, and only a few vesicles were scattered in the network of tonofilaments (Fig. 7a). The tonofilaments were packed more densely, but their random reticular arrangement persisted. The percentage of cell border occupied by desmosomes had decreased to 10 ± 1.6.

In the experimental animals, little change other than the increased opacity of the cell membrane and the disappearance of MCG characterized the transition between granular and horny layer. Most cells retained their corrugated outline and round shape. Nuclei persisted. They were of smaller size than in the granular layer, and the nuclear membrane was not present. The nucleoplasm consisted of granular material of variably dense packing. Numerous RNP particles could be identified in the cytoplasm. KHG, of diminished size and irregular shape, were frequent in the lower horny layers, and less frequent towards the surface. Some showed surface depressions associated with vesicles (Fig. 6b). Vesicles persisted throughout the horny layer and were far more numerous than in the controls. Clumps of granular material distinct from nuclear remnants or KHG and associated with vesicular structures persisted in the outermost layers (Fig. 7b). The percentage of cell border occupied by desmosomes had increased to 25 ± 1.6 and was significantly higher than in the controls.

2. *Palate.* As in cheek, no consistent differences between control and experimental animals were noted deep to the upper spinous layer.

Upper spinous layer (Fig. 8a, b). In the upper spinous layer, no experimental changes in the number of RNP particles or mitochondria were noted. In the more superficial spinous cells, the experimental animals possessed thinner and fewer bundles of tonofilaments than the controls. MCG were present in smaller number than in the controls.

No changes in nuclear morphology were noted.

Granular layer (Fig. 9a, b). As at the preceding level, no differences between experimental and control animals were noted in numbers of RNP particles and of mitochondria. The experimental differences noted in the upper spinous cells grew more prominent with outward progression. The bundles of tonofilaments in the experimental animals were fewer and smaller and the individual filaments more loosely packed. The experimental animals possessed markedly fewer MCG. The most striking experimental change was shown by the KHG. Whereas in the controls the number and size of KHG grew sharply, in the experimental animals only a few, consistently small KHG were present throughout the granular layer. No changes in nuclear morphology were noted. The percentages of desmosomes were 31 ± 1.8 in the controls and 27 ± 1.4 in the experimental animals.

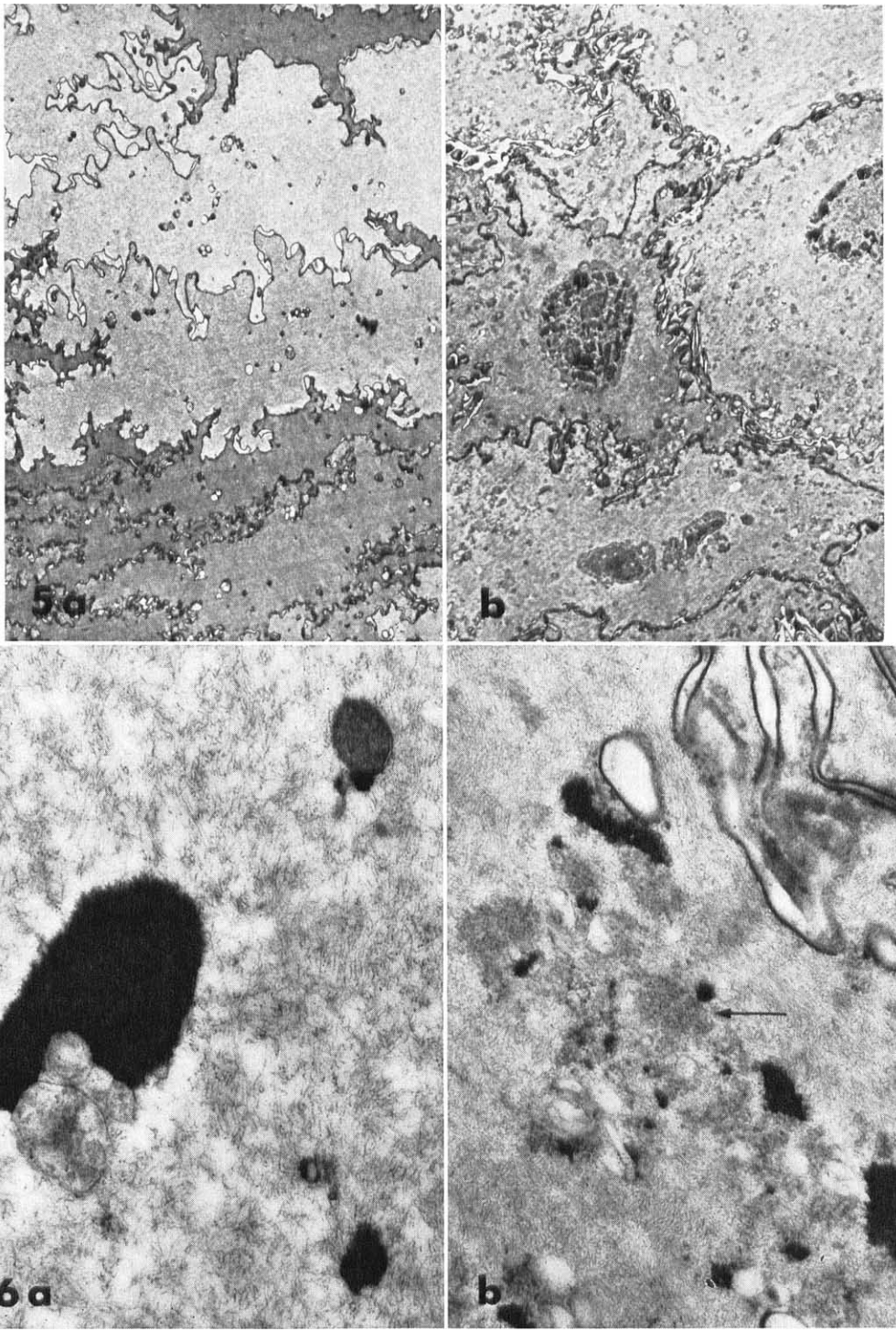
Keratin layer, deeper part (Fig. 10a, b). The transition from granular to keratin layers in the controls was characterized by (1) markedly greater flattening of the cells, (2) increased stainability of the plasma membrane, (3) increased electron opacity of the cytoplasm, hence little contrast in opacity of cytoplasm and plasma membrane, (4) the disappearance of nuclei from all but the deepest layer, (5) the disappearance of mitochondria, RNP particles, MCG, intracellular membrane systems and KGH, and (6) the disappearance of individually resolvable tonofilaments due to their tighter packing and the presence of an embedding matrix.

In common with the control animals, the experimental animals showed flattening of cells, a sudden increase in the stainability of the plasma membrane, and disappearance of MCG at the transition to the keratin layer. In contrast to the control keratin, the experimental keratin showed no increase in electron opacity

Figures 3 through 9 are electron micrographs of buccal mucosa from control and experimental animals. The illustrations are taken from specimens from animals killed after eight weeks on their respective diets.

FIG. 3. a) Control; b) experimental (× 29,270). Center of spinous layer. Illustrates in both control and experimental animal similar density of tonofilaments and persistent diffuse distribution. Note absence of experimental differences in nucleus but closer spacing of mitochondria and groups of RNP particles in the experimental animal.

FIG. 4. a) Control; b) experimental (× 29,500). Center of granular layer. Illustrates in both groups the presence of KHG of regular shape, their association with RNP particles, and the lack of association with the diffusely distributed tonofilaments. Note association of KHG to endoplasmic reticulum and mitochondria in a and b (↑).



FIGS. 5 AND 6.

of the cytoplasm compared to that of the granular cells. Hence, the cytoplasm was much less opaque than the plasma membrane. Nuclear remnants were present throughout, consisting of a granular material that was variably dense in packing. The nuclei were smaller than in the granular layer and the nuclear membrane was absent. RNP particles and vesicular structures persisted. The tonofilaments persisted as bundles, were less tightly packed than in the controls and did not appear to be embedded in a matrix; thus the individual filaments could be resolved. The bundles were separated by spaces which usually appeared "empty," but frequently contained RNP particles or vesicular structures. The desmosome percentages had increased in control and experimental animals, but far more in the latter. They were 43 ± 5.1 in controls and 83 ± 3.0 in the experimental animals.

Superficial keratin layers (Fig. 11a, b). In the control animals, no changes were distinguishable between lower and superficial keratin, except for the now complete absence of nuclear remnants. In the experimental keratin, perceptible changes took place with outward progression. The tonofilaments became more tightly packed and the amount of "empty" space decreased, but no embedding matrix was noted. Nuclear remnants, RNP particles, and vesicular structures persisted but decreased in amount.

DISCUSSION

Experimental Response of Cheek and Palate Mucosa

In buccal mucosa, mild as well as severe zinc deprivation caused a parakeratotic response in the epithelium of the whole region. In palatal mucosa, mild zinc deprivation caused no parakeratotic response, severe deprivation at most caused a response confined to the epithelium between rugae. This differential effect of zinc-low diets is one of many differences in the biochemistry (11) and morphology (10, 12, 13) of rodent buccal and palatal mucosa shown in previous studies.

Common features in parakeratinized mucosa of cheek and palate were the absence of experimental changes in lamina propria and lower cell layers and the persistence in the keratin of cytoplasmic organelles normally not visible beyond the granular layer. Furthermore, in both regions, the nucleus, though persisting in the experimental keratin while disappearing in the control keratin, showed no experimental differences in any of the cellular layers.

Experimental Effects on the Concentration of Cytoplasmic Organelles

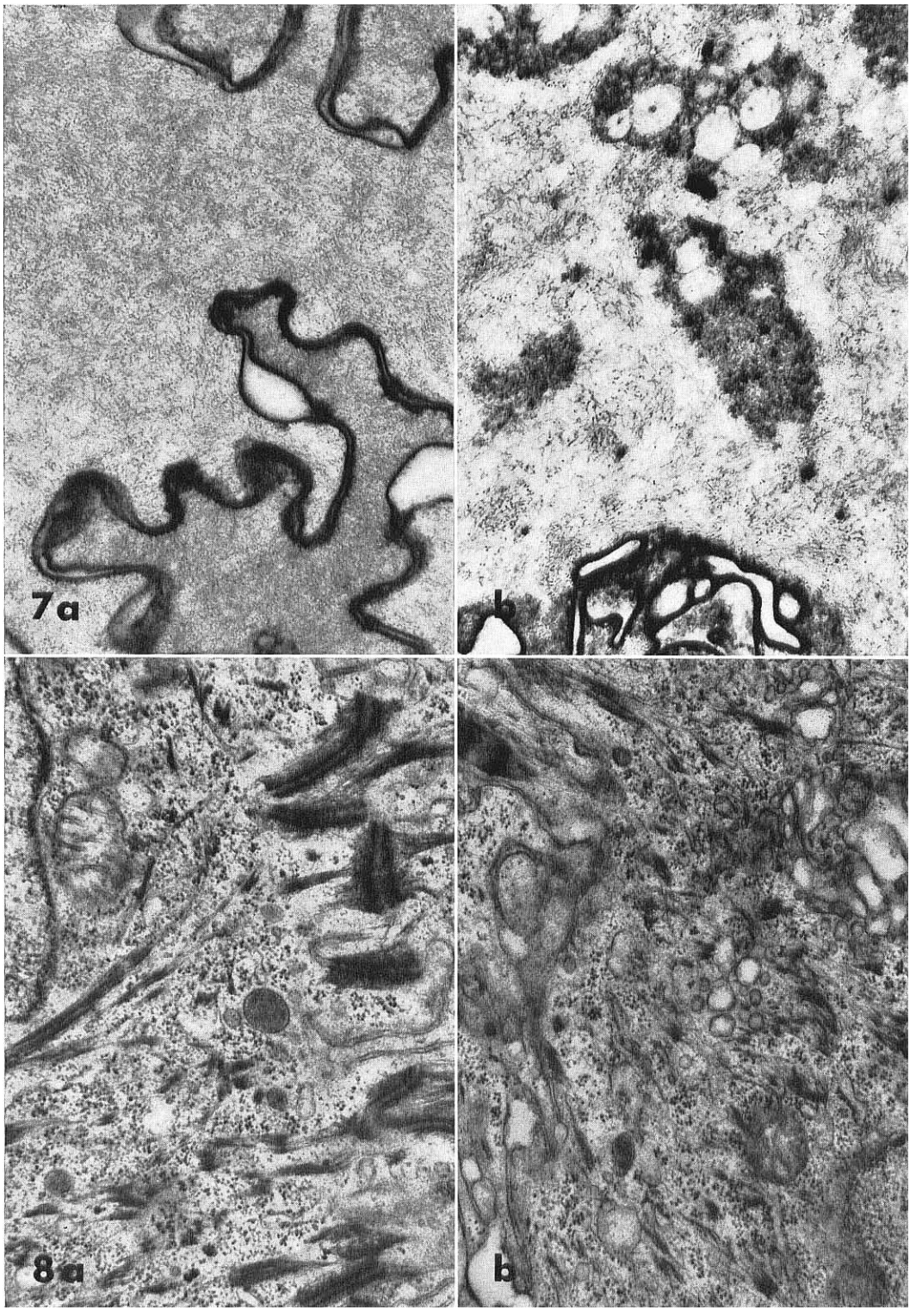
The cytoplasmic changes demonstrable in the upper cellular and keratin layers of experimental cheek and palate were not identical. The experimental effects in the *upper cellular layers* are summarized in Table III. The table shows that the buccal and palatal response differed for all cytoplasmic organelles for which estimates of concentration changes had been made.

Comparison shows that in *control cheek and palate*, the cytoplasmic organelles undergo divergent changes in concentration with maturation of the cells. Both epithelia are rapidly renewing tissues, and in both, the cells undergo a marked increase in size before they reach the keratin layer. In *palate*, upper spinous cells have about four times the volume of basal cells, granular cells have about six times the volume of basal cells (14). With peripheral progression, mitochondria, free ribosomes and endoplasmic reticulum showed hardly any decrease in concentration. The concentration of tonofilaments increased progressively and MCG and KHG appeared, indicating that synthetic processes continued in the upper cells. This conclusion is confirmed by the finding of a

Figures 5 through 7 are electron micrographs of the keratin layer

FIG. 5. a) Control; b) experimental (× 3,240). Survey view, center of keratin layer. Note flattened cell shape, absence of nuclear remnants and the small number of vesicular structures in a, in contrast to the round cell shape, persistence of nuclei and the presence of numerous vesicles in b.

FIG. 6. a) Control; b) experimental (× 29,500). Lower keratin layer. Illustrates the similarity in the packing and arrangement of tonofilaments, the irregular shape of KHG, and their association with vesicular structures in control and experimental animal. However, note the persistence of greater numbers of both of these structures in b and the presence of a granular material (↑) which is not seen in a.



FIGS. 7 AND 8.

progressively increasing dry weight per cell (14). In the *experimental palate,* increase of cell size with peripheral progression was comparable to that in controls. No experimental changes in concentration were noted in mitochondria, free ribosomes and endoplasmic reticulum. As in the controls, these elements of cellular synthetic machinery were present in unchanging concentrations throughout the cellular layers. The products of synthesis, however, did not show the increase in concentration noted with peripheral progression in the controls. Hence, tonofilaments, MCG, and KHG all showed lower concentrations in the upper cellular layers of experimental specimens than in those of controls.

In *control cheek* in contrast to control palate the concentrations of mitochondria, free ribosomes and endoplasmic reticulum decreased rapidly superficial to the lower spinous cells. No increase or decrease occurred in the concentration of tonofilaments, and MCG and KHG appeared in upper spinous and granular cells. These products of synthesis, especially KHG, remained sparse compared to their numbers in the palate. However, the daily rate of growth is 2.6 times that in the palate (as estimated in the mouse, 12), and the peripheral increase in cell size and weight is much greater than in the palate (14). The volume of upper spinous cells is 10 times that of basal cells, and the volume of granular cells 30 times that of basal cells. The dry mass per cell increases nearly in proportion to the increase in volume. Granular cells weigh 28 times as much as basal cells. It is clear that synthetic processes continue in upper buccal cells also. The decreasing concentrations of mitochondria and ribosomes are most likely due to their dispersal in larger cells.

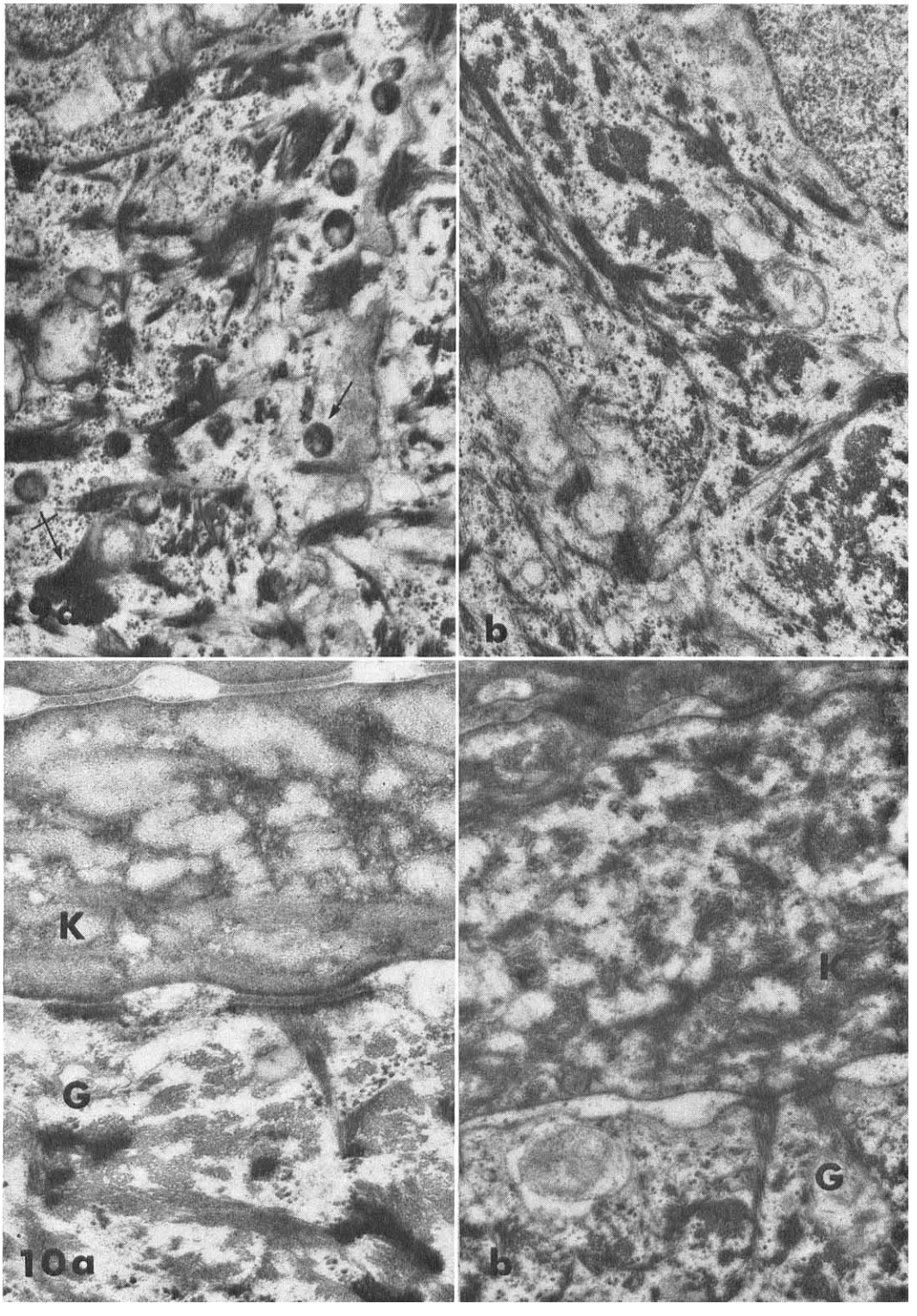
In the *experimental cheek,* mitochondria, free ribosomes and endoplasmic reticulum did not show the rapid decreases in concentration observed with peripheral progression in the controls. Hence, their concentration in the upper spinous and granular layers far exceeded that in the controls. The greater concentration of ribosomes was equally striking in Alvares and Meyer's light microscopic observations (2). Growth in cell size was comparable in the experimental and control animals, hence, dispersal of organelles due to increasing cytoplasmic volume should have operated to the same degree in both groups. The fact that the concentration of elements of synthetic machinery did not decrease in the experimental animals is most likely the result of their continued formation. While cellular machinery for synthesis was present in greater concentration in the experimental than in the control specimens, the *products* of synthesis showed no corresponding experimental increase. The experimental cheek showed no difference from control cheek in the concentrations of tonofilaments and MCG, and a slight decrease in the concentration of KHG.

It is commonly held that there is a quantitative relationship between the synthetic machinery and the synthetic activity of a cell (15). This relation seemed altered in both experimental cheek and palate. In cheek, the change was from increased machinery to unchanged quantity of product, in the palate from unchanged machinery to decreased quantity of product. The effect of Zn-deficiency common to both regions appears to be a decreased efficiency of the synthetic machinery. A relation between zinc-deficiency and impaired ribosomal biological activity may perhaps be inferred from the work of Vallee's group (16). Such impairment could be the cause of the disturbance in both tissues. It is not clear why buccal but not palatal epithelium responded with increased formation of the organelles for synthesis.

FIG. 7. a) Control; b) experimental (× 29,500). Upper keratin layer. Note the absence of structures other than the tonofilaments in the control animal in contrast to the persistence of KHG and numerous vesicular structures, which are associated with clumps of granular material, in the experimental animal.

Figures 8 through 11 are electron micrographs of palatal mucosa from control and experimental animals. The illustrations used for the experimental animal are taken from the most severely affected animal in the group killed after 8 weeks on the diet.

FIG. 8. a) Control; b) experimental (× 29,500). Upper spinous layer. Illustrates similarities in the distribution of mitochondria and RNP particles and the organization of the tonofilaments into bundles in both a and b. Note the association of tonofilaments with desmosomes in both but reduced number and size of desmosomes and bundles of tonofilaments in b.



FIGS. 9 AND 10.

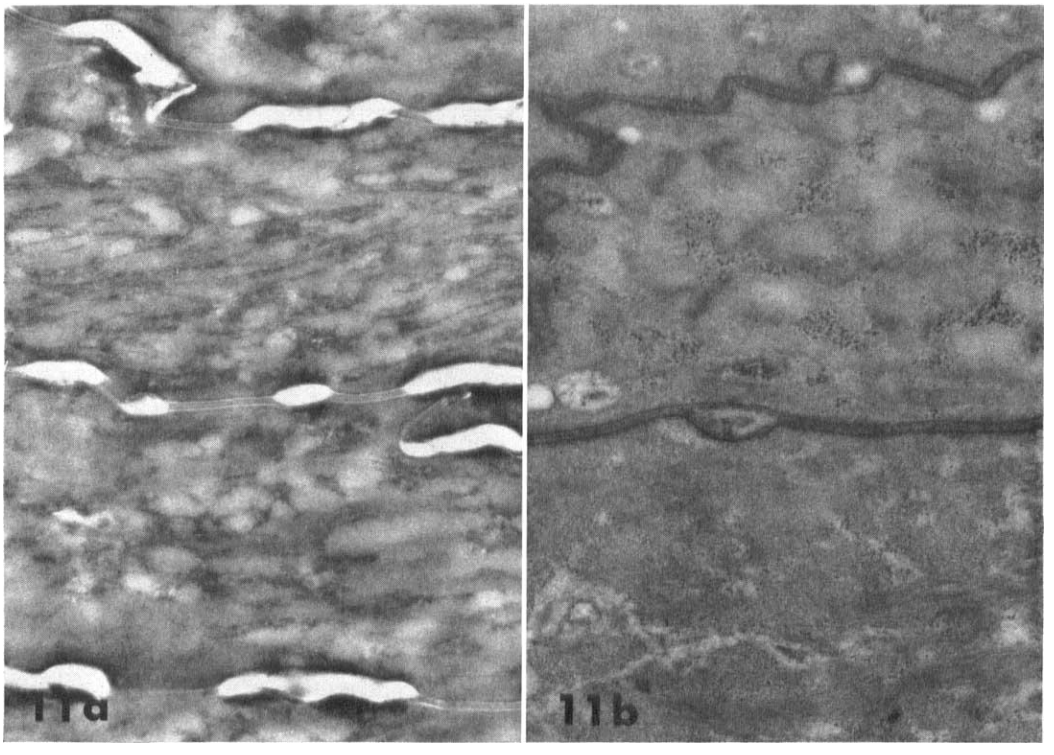


FIG. 11. a) Control; b) experimental (× 29,500). Upper keratin layer. Note in a the similarity in the opacity of cytoplasm and plasma membrane but in b their persistent contrast in stainability; note further the strikingly greater proportion of plasma membrane occupied by desmosomes in this animal. Note in the control animal the similarity between lower and upper keratin (10a and 11a) and in the experimental animal the marked contrast between these two levels (10b and 11b). Fig. 11b illustrates the decrease in "empty" space and vesicular structures and the tighter packing of tonofilaments in the superficial compared to the deeper experimental keratin layer. The area does not demonstrate the concomitant decrease in RNP particles typically seen in superficial compared to deeper experimental keratin.

Experimental Effects on Lytic Processes

The present findings point to a more generalized failure of lytic processes than has hitherto been ascribed to zinc deficiency. Follis *et al.* (1) showed that zinc-deficiency causes the persistence of nuclei in normally orthokeratinizing epithelia. The present study has shown that most cytoplasmic organelles which normally disappear as abruptly as the nucleus persist likewise. The pertinent findings are summarized in Table IV. In the keratin of *experimental cheek*, nuclear chromatin, ribosomes, mitochondria and endoplasmic reticulum showed no changes in concentration between granular layer and keratin and KHG only a slight decrease in number and size. Of the nor-

FIG. 9. a) Control; b) experimental (× 29,500). Middle granular layer. Illustrates comparable numbers of mitochondria and RNP particles, and similar arrangement of tonofilaments into bundles in a and b. Note in the experimental animal the fewer and smaller bundles of tonofilaments, fewer round bodies (↑) and the absence of KHG (↑).

FIG. 10. a) Control; b) experimental (× 29,500). Transition zone, granular (G)—keratin (K) layers. Illustrates in the granular cells the smaller bundles of tonofilaments and total absence of KHG in the experimental animal. In the lowest keratinized cells of both a and b note the marked increase in stainability of the plasma membrane compared to that in the most superficial granular cell but also note the marked difference in the cell residues of a and b: the persistence in the experimental animal of RNP particles, vesicular structures, and of discrete bundles of tonofilaments separated by "empty" spaces.

mal disintegrative processes at the transition from granular to keratin layer, only those leading to the disappearance of MCG and of the nuclear membrane and to a partial disintegration of KHG took place. In the *experimental palate*, disintegrative changes at the granular-keratin junction were likewise in the main limited to the MCG and nuclear membranes. In cheek, the failure of disintegrative processes affected the entire keratin layer; in the superficial keratin of the palate, nuclear remnants became smaller and less common than in the deep keratin, and free ribosomes, mitochondrial residues, and endoplasmic reticulum were reduced in number (Table V).

The normal breakdown of cellular structures at the granular-keratin junction has to date not been convincingly clarified. The organelles effecting this disintegration may not be the same in epithelia as different as those of cheek and palate. In mouse mucosa of the buccal type and in mouse epidermis, structures resembling kidney lysosomes have been demonstrated in electron microscope preparations treated to show acid phosphatase and arylsulfatase (17). The present findings suggest in both control and experimental rat buccal mucosa the association of mitochondrial residues with lytic processes, showing such residues in depressions of KHG of reduced size and irregular shape. In rat mucosa of palatal type acid phosphatase activity was seen in the Golgi zone, in various-sized vesicles and in a shell surrounding mitochondrial remnants (18, 19). Diffuse acid phosphatase activity at the junction of granular and keratin layers appears to

TABLE III

Experimental changes in the concentration of cell organelles in upper spinous and granular layers

Organelle	Concentration compared to controls	
	Cheek	Palate
Mitochondria	Increased	Unchanged
Free ribosomes	Increased	Unchanged
Endoplasmic reticulum	Increased	Unchanged
Tonofilaments	Unchanged	Decreased
KHG	Slightly decreased	Markedly decreased
Round bodies (MCG)	Unchanged	Decreased

TABLE V

Changes in concentration of cell organelles occurring between deep and superficial keratin of the experimental animals

Organelle	Change between deep and superficial keratin	
	Cheek	Palate
Nucleus	No change	No change (decrease in size)
Free ribosomes	No change	Decrease
Mitochondria, double walled vesicles	No change	Decrease
Endoplasmic reticulum, single walled vesicles	No change	Decrease

TABLE IV

Changes in concentration of cell organelles occurring between granular layer and deeper portion of keratin

Organelle	Change between granular layer and keratin			
	Cheek		Palate	
	Experimental	Control	Experimental	Control
Nucleus	No change	Disappearance	No change	Disappearance
Free ribosomes	Slight decrease	Disappearance	Slight decrease	Disappearance
Mitochondria, double walled vesicles	Slight decrease	Marked decrease	Slight decrease	Disappearance
Endoplasmic reticulum, singled-walled vesicles	Slight decrease	Marked decrease	Slight decrease	Disappearance
KHG	Slight decrease	Marked decrease	No change	Disappearance
MCG	Disappearance	Disappearance	Disappearance	Disappearance

occur in many types of keratinizing epithelia (17, 20, 21, 22, 23). Investigation of the effects of zinc deficiency on cell organelles treated to show lysosomal activity might help to clarify the lytic processes in the normal animal.

Behavior of Desmosomes in the Keratin Layer

Both control and experimental specimens of *palate* showed a rise in the percentage of cell border occupied by desmosomes in the keratin compared to the granular cells. Since the keratin cells were smaller than the granular cells, this rise could be due to the reduction in surface area rather than the formation of new attachment plaques.

In the controls, cells in granular and keratin layers were similar in shape and in smoothness of contour. Cell volume in the keratin averaged a third that in granular cells (14), which would correspond to a reduction in circumference by roughly 30%. The percentage of cell border occupied by desmosomes rose by about 40%, from 31% in the granular layer to 43% in the keratin. Thus no appreciable new formation of desmosomes has to be postulated to account for their rising proportion in the keratin layer.

In the experimental specimens, inspection of size and contour suggested a slighter reduction of cell circumference than in controls. The percentage of desmosomes rose from 27 in the granular layer to 83 in the keratin layer. This more than threefold rise is entirely out of proportion to any possible reduction in cell circumference, suggesting that new attachment plaques must have been formed in the palatal keratin layer of the zinc-deficient animals.

Buccal keratin of experimental animals also showed a significantly higher percentage of cell border occupied by desmosomes than in controls (25% in the experimental animals, 10% in the controls). However, in both groups the irregularities of cell contour precluded evaluation of the changes in cell circumference between granular and keratin layer.

Comparison of Ultrastructural Changes in Psoriasis and Zinc Deficiency

Psoriasis is the most common cause of parakeratosis of the epidermis (24, 25) and has been the subject of extensive electron microscope study. Since parakeratosis induced by zinc deficiency has been proposed as a model for clinical states of parakeratinization, it seemed interesting to compare the reported ultrastructural changes in psoriasis with those in zinc deficiency. There seem to be some major differences and some points of similarity in the two parakeratotic conditions. One difference is in the level at which changes from the normal occur. In psoriatic skin, Brody (26, 27) has described ultrastructural changes in the subepithelial tissue as well as in the basal and lower spinous cells, whereas in zinc deficiency the first noticeable changes in cheek and palate mucosa occurred in the upper spinous layers.

All three tissues show quantitative changes from the normal with respect to cellular synthetic machinery and synthesized product, but the changes are not identical ones. With respect to synthetic machinery in the upper cellular layers, psoriatic skin behaves like cheek epithelium, showing increases over normal skin in RNP particles, mitochondria and endoplasmic reticulum (27, 28, 29, 30). With respect to products of synthesis, however, the changes are like those in the palate epithelium, consisting in decreases in tonofilaments, MCG, and KHG. Thus psoriatic skin like zinc-deficient cheek and palate shows a disturbance in the normal quantitative relation between synthetic machinery and synthesized products. The disturbance seems to be more severe in psoriasis than in zinc deficiency, since in psoriasis a decrease in product occurs *despite* an increase in machinery. This more severe disturbance might be related to the more marked increase in the rate of cell division. Whereas 27-fold increases have been reported in psoriasis (31), a five-fold increase is the maximum reported in zinc deficiency (2).

With respect to disintegrative processes, all three tissues show disturbances beyond the failure of nuclear disintegration. In psoriasis as well as in zinc deficiency, RNP particles, mitochondria and endoplasmic reticulum persist in the keratin layer. In psoriasis, MCG persist as well (29), but in zinc deficiency, these are absent in buccal and palatal keratin. A further difference is the reduction in desmosomes reported for psoriatic keratin (29, 32) in contrast to their marked increase in zinc-deficient cheek and palate keratin.

A more definitive evaluation of zinc deficiency-induced parakeratosis as a model for clinical conditions of parakeratosis awaits the

study of the ultrastructure of *epidermal* lesions in experimentally induced zinc deficiency.

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